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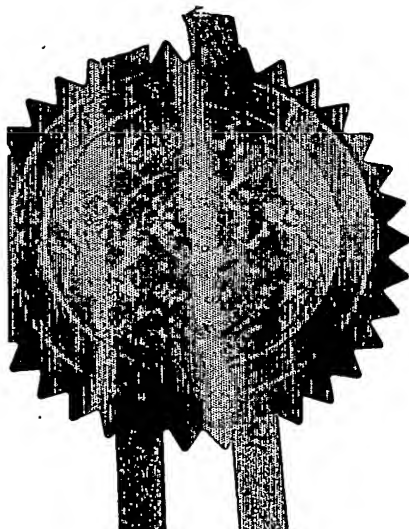
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Andrew Gersey

Dated

10 September 2003



The
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1/77

24OCT02 E758078-1 D01030
P01/7700 0.00-0224677.5

Request for grant of a patent

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The Patent Office
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1. Your reference

MG/PMS/P33130

2. Patent application number

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23 OCT 2002

0224677.5

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Glaxo Group Limited
Glaxo Wellcome House, Berkeley Avenue,
Greenford, Middlesex UB6 0NN, Great Britain

Patents ADP number (*if you know it*) 00473587003

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Novel Compounds

5. Name of your agent (*if you have one*)

Corporate Intellectual Property

"Address for service" in the United Kingdom to which all correspondence should be sent
(including the postcode)

GlaxoSmithKline
Corporate Intellectual Property (CN9 25.1)
980 Great West Road
BRENTFORD
Middlesex TW8 9GS

Patents ADP number (*if you know it*)

07960982003

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (*if you know it*) the or each application number

Country	Priority application number (<i>if you know it</i>)	Date of filing (<i>day / month / year</i>)
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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (<i>day / month / year</i>)
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (*Answer yes if:*

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is named as an applicant, or
 - c) any named applicant is a corporate body
- See note (d)

Patents Form 1/77

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Description	12
Claim(s)	2
Abstract	0
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Priority Documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(please specify)

11.

We request the grant of a patent on the basis of this application

Signature

J N Rice

Date 23-Oct-02

12. Name and daytime telephone number of person to contact in the United Kingdom

J N Rice 01279 644508

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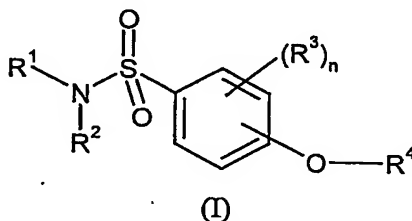
Novel Compounds

The present invention relates to novel benzenesulfonamide derivatives having pharmacological activity, processes for their preparation, to compositions containing them and to their use in the treatment of neurological and psychiatric disorders.

WO 00/06254 (Societe Civile Bioprojet) describe a series of compounds which are claimed to be histamine H3 antagonists.

The histamine H3 receptor is predominantly expressed in the mammalian central nervous system (CNS), with minimal expression in peripheral tissues except on some sympathetic nerves (Leurs *et al.*, (1998), Trends Pharmacol. Sci. 19, 177-183). Activation of H3 receptors by selective agonists or histamine results in the inhibition of neurotransmitter release from a variety of different nerve populations, including histaminergic and cholinergic neurons (Schlicker *et al.*, (1994), Fundam. Clin. Pharmacol. 8, 128-137). Additionally, *in vitro* and *in vivo* studies have shown that H3 antagonists can facilitate neurotransmitter release in brain areas such as the cerebral cortex and hippocampus, relevant to cognition (Onodera *et al.*, (1998), In: The Histamine H3 receptor, ed Leurs and Timmerman, pp255-267, Elsevier Science B.V.). Moreover, a number of reports in the literature have demonstrated the cognitive enhancing properties of H3 antagonists (e.g. thioperamide, clobenpropit, ciproxifan and GT-2331) in rodent models including the five choice task, object recognition, elevated plus maze, acquisition of novel task and passive avoidance (Giovanni *et al.*, (1999), Behav. Brain Res. 104, 147-155). These data suggest that novel H3 antagonists such as the current series could be useful for the treatment of cognitive impairments in diseases such as Alzheimer's disease and related neurodegenerative disorders.

The present invention provides, in a first aspect, a compound of formula (I) or a pharmaceutically acceptable salt thereof:

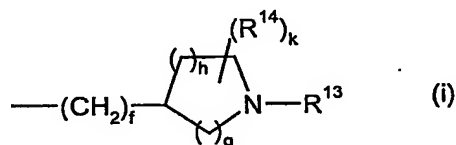


wherein:

NR¹R² represents an N-linked -heterocyclyl, -heterocyclyl-X-aryl, -heterocyclyl-X-heteroaryl, -heterocyclyl-X-heterocyclyl, -heteroaryl, -heteroaryl-X-aryl, -heteroaryl-X-heteroaryl or -heteroaryl-X-heterocyclyl group;

wherein said aryl, heteroaryl and heterocyclyl groups of NR¹R² may be optionally substituted by one or more (eg. 1, 2 or 3) substituents which may be the same or different, and which are selected from the group consisting of halogen, hydroxy, cyano, nitro, oxo, haloC₁₋₆ alkyl, polyhaloC₁₋₆ alkyl, haloC₁₋₆ alkoxy, polyhaloC₁₋₆ alkoxy, C₁₋₆ alkyl, C₁₋₆ alkoxy, arylC₁₋₆ alkoxy, C₁₋₆ alkylthio, C₁₋₆ alkoxyC₁₋₆ alkyl, C₃₋₇ cycloalkylC₁₋₆ alkoxy, C₁₋₆ alkanoyl, C₁₋₆ alkoxycarbonyl, arylC₁₋₆ alkyl, heteroarylC₁₋₆ alkyl, C₁₋₆ alkylsulfonyl, C₁₋₆ alkylsulfinyl, C₁₋₆

alkylsulfonyloxy, C₁₋₆ alkylsulfonylC₁₋₆ alkyl, arylsulfonyl, arylsulfonyloxy, arylsulfonylC₁₋₆ alkyl, aryloxy, C₁₋₆ alkylsulfonamidoC₁₋₆ alkyl, C₁₋₆ alkylamidoC₁₋₆ alkyl, arylsulfonamido, arylaminosulfonyl, arylsulfonamidoC₁₋₆ alkyl, arylcarboxamidoC₁₋₆ alkyl, aroylC₁₋₆ alkyl, arylC₁₋₆ alkanoyl, or a group -NR¹⁵R¹⁶, -CONR¹⁵R¹⁶, -NR¹⁵COR¹⁶, -NR¹⁵SO₂R¹⁶ or -SO₂NR¹⁵R¹⁶, wherein
 5 R¹⁵ and R¹⁶ independently represent hydrogen or C₁₋₆ alkyl;
 X represents a bond, CO, NHCO or CONH;
 R³ represents halogen, C₁₋₆ alkyl, C₁₋₆ alkoxy, cyano, amino or trifluoromethyl;
 n is 0, 1 or 2;
 R⁴ represents -(CH₂)_q-NR¹¹R¹² or a group of formula (i):



wherein q is 2, 3 or 4;

R¹¹ and R¹² independently represent C₁₋₆ alkyl or together with the nitrogen atom to which they
 15 are attached represent an N-linked heterocyclic group optionally substituted by one or two R¹⁷ groups;

R¹³ represents hydrogen, C₁₋₆ alkyl, C₃₋₈ cycloalkyl, -C₁₋₆ alkyl-aryl or heterocyclyl;

R¹⁴ and R¹⁷ independently represent halogen, C₁₋₆ alkyl, haloC₁₋₆ alkyl, OH, diC₁₋₆ alkylamino or C₁₋₆ alkoxy;

f and k independently represent 0, 1 or 2;

g is 0, 1 or 2 and h is 0, 1, 2 or 3, such that g and h cannot both be 0;
 or solvates thereof.

Alkyl groups, whether alone or as part of another group, may be straight chain or branched and
 25 the groups alkoxy and alkanoyl shall be interpreted similarly. Alkyl moieties are more preferably C₁₋₄ alkyl, eg. methyl or ethyl. The term 'halogen' is used herein to describe, unless otherwise stated, a group selected from fluorine, chlorine, bromine or iodine.

The term "aryl" includes phenyl and naphthyl.

The term "heterocyclyl" is intended to mean a 4-7 membered monocyclic saturated or partially
 unsaturated aliphatic ring or a 4-7 membered monocyclic saturated or partially unsaturated
 aliphatic ring fused to a benzene ring containing 1 to 3 heteroatoms selected from oxygen or
 nitrogen. Suitable examples of such monocyclic rings include pyrrolidinyl, piperidinyl,
 35 piperazinyl, morpholinyl, diazepanyl and azepanyl. Suitable examples of benzofused heterocyclic
 rings include indolinyl, isoindolinyl and tetrahydroisoquinolinyl.

The term "heteroaryl" is intended to mean a 5-7 membered monocyclic aromatic or a fused 8-11
 40 membered bicyclic aromatic ring containing 1 to 3 heteroatoms selected from oxygen, nitrogen
 and sulphur. Suitable examples of such monocyclic aromatic rings include thienyl, furyl,

pyrrolyl, triazolyl, imidazolyl, oxazolyl, thiazolyl, oxadiazolyl, isothiazolyl, isoxazolyl, thiadiazolyl, pyrazolyl, pyrimidyl, pyridazinyl, pyrazinyl and pyridyl. Suitable examples of such fused aromatic rings include benzofused aromatic rings such as quinoliny, isoquinoliny, quinazolinyl, quinoxaliny, cinnoliny, naphthyridiny, indolyl, indazolyl, pyrrolopyridiny, benzofuranyl, benzothienyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzothiazolyl, benzisothiazolyl, benzoxadiazolyl, benzothiadiazolyl and the like.

Preferably, NR^1R^2 represents an N-linked heterocyclyl (eg. morpholiny, piperidiny, indoliny, isoindoliny or piperazinyl) or a -heterocyclyl-X-aryl group (eg. -piperidiny-phenyl, -piperazinyl-phenyl, -piperazinyl-CO-phenyl or -piperazinyl-CO-naphthyl) optionally substituted by a polyhalo C_{1-6} alkoxy (eg. trifluoromethoxy) group.

Preferably, n represents 0.

Preferably, R^4 represents $-(CH_2)_q-NR^{11}R^{12}$.

Preferably, q is 3.

Preferably, $NR^{11}R^{12}$ represents an N-linked heterocyclic group, more preferably unsubstituted piperidine.

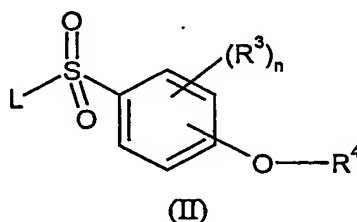
Preferred compounds according to the invention include examples E1-E9 as shown below, or a pharmaceutically acceptable salt thereof.

Compounds of formula (I) may form acid addition salts with acids, such as conventional pharmaceutically acceptable acids, for example maleic, hydrochloric, hydrobromic, phosphoric, acetic, fumaric, salicylic, sulphate, citric, lactic, mandelic, tartaric and methanesulphonic. Salts, solvates and hydrates of histamine H3 receptor antagonists therefore form an aspect of the invention.

Certain compounds of formula (I) are capable of existing in stereoisomeric forms. It will be understood that the invention encompasses all geometric and optical isomers of these compounds and the mixtures thereof including racemates. Tautomers also form an aspect of the invention.

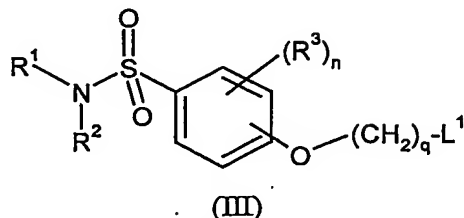
The present invention also provides a process for the preparation of a compound of formula (I) or a pharmaceutically acceptable salt thereof, which process comprises:

(a) reacting a compound of formula (II)



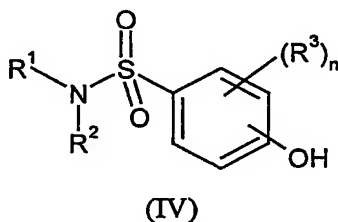
with a compound of formula R^1R^2NH wherein R^1 , R^2 , R^3 , n and R^4 are as defined above and L is OH or a suitable leaving group (eg. a halogen atom such as chlorine); or

(b) preparing a compound of formula (I) wherein R^4 represents $-(CH_2)_q-NR^{11}R^{12}$ which comprises reacting a compound of formula (III)



wherein R^1 , R^2 , R^3 , n and q are as defined above and L^1 represents a suitable leaving group such as a halogen atom (eg. bromine) with a compound of formula $HNR^{11}R^{12}$; wherein R^{11} and R^{12} are as defined above; or

10 (c) reacting a compound of formula (IV)



15 wherein R^1 , R^2 , R^3 and n are as defined above, with a compound of formula R^4-L^2 , wherein R^4 is as defined above and L^2 represents a suitable leaving group such as a halogen atom or an OH group; or

(d) deprotecting a compound of formula (I) which is protected; and optionally thereafter

20 (e) interconversion to other compounds of formula (I).

Process (a) typically comprises reaction with the compound of formula R^1R^2NH optionally in the presence of a suitable base such as triethylamine or a solid supported amine, in a suitable solvent such as dichloromethane. When L represents OH, process (a) typically comprises an initial halogenation reaction of the compound of formula (II) with a suitable halogenating agent (eg. thionyl chloride) prior to reaction with the compound of formula R^1R^2NH as above.

Process (b) is typically performed in the presence of a suitable solvent (such as 1-butanol) at an elevated temperature.

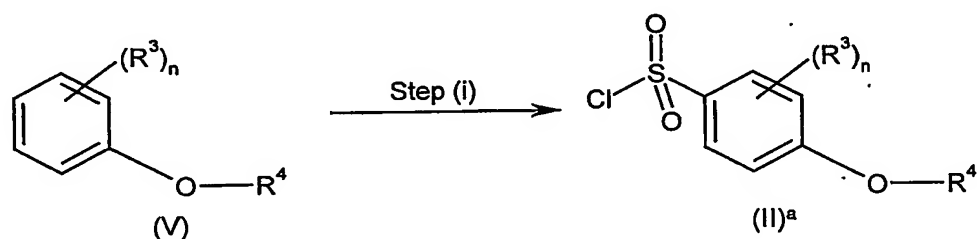
Process (c) typically comprises an alkylation reaction under Mitsunobu conditions.

In process (d), examples of protecting groups and the means for their removal can be found in T. W. Greene 'Protective Groups in Organic Synthesis' (J. Wiley and Sons, 1991). Suitable amine protecting groups include sulphonyl (e.g. tosyl), acyl (e.g. acetyl, 2',2',2'-trichloroethoxycarbonyl,

benzyloxycarbonyl or t-butoxycarbonyl) and arylalkyl (e.g. benzyl), which may be removed by hydrolysis (e.g. using an acid such as hydrochloric acid) or reductively (e.g. hydrogenolysis of a benzyl group or reductive removal of a 2',2',2'-trichloroethoxycarbonyl group using zinc in acetic acid) as appropriate. Other suitable amine protecting groups include trifluoroacetyl (-COCF₃) which may be removed by base catalysed hydrolysis or a solid phase resin bound benzyl group, such as a Merrifield resin bound 2,6-dimethoxybenzyl group (Ellman linker), which may be removed by acid catalysed hydrolysis, for example with trifluoroacetic acid.

Process (e) may be performed using conventional interconversion procedures such as epimerisation, oxidation, reduction, alkylation, nucleophilic or electrophilic aromatic substitution, ester hydrolysis or amide bond formation.

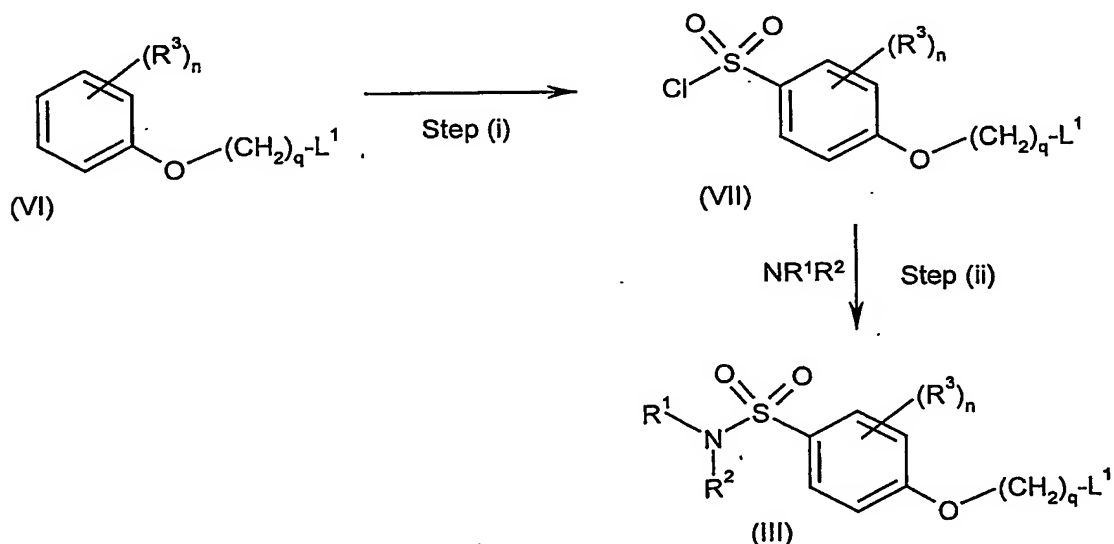
Compounds of formula (II) wherein L represents chlorine may be prepared in accordance with the following procedure:



wherein R³, R⁴ and n are as defined above.

Step (i) typically comprises reaction of a compound of formula (V) with a suitable reagent such as chlorosulfonic acid in a suitable solvent such as chloroform.

Compounds of formula (III) may be prepared in accordance with the following procedure:

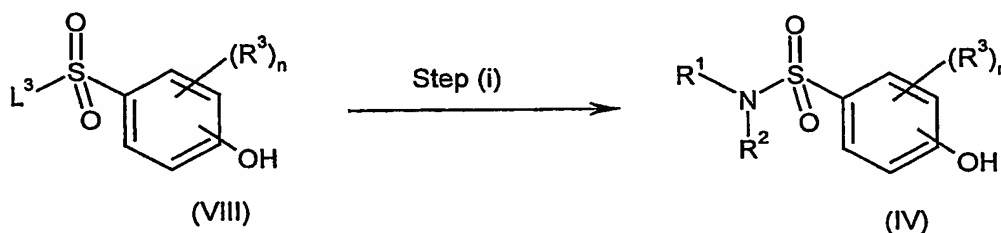


wherein R³, n, q, L¹, R¹ and R² are as defined above.

Step (i) may be performed by reacting a compound of formula (VI) with a suitable reagent such as chlorosulfonic acid in a suitable solvent such as chloroform.

- 5 Step (ii) is typically performed in the presence of a suitable solvent such as dichloromethane.

Compounds of formula (IV) may be prepared in accordance with the following procedure:



- 10 wherein R^1 , R^2 , R^3 and n are as defined above and L^3 represents a suitable leaving group such as a halogen atom (eg. chlorine).

- 15 Step (i) typically comprises reaction of a compound of formula (VIII) with a compound of formula R^1R^2NH , wherein R^1 and R^2 are as defined above, in a suitable solvent such as dichloromethane.

Compounds of formula R^1R^2NH , (V), (VI) and (VIII) are either known in the literature or can be prepared by analogous methods.

- 20 Compounds of formula (I) and their pharmaceutically acceptable salts have affinity for the histamine H3 receptor and are believed to be of potential use in the treatment of neurological diseases including Alzheimer's disease, dementia, age-related memory dysfunction, mild cognitive impairment, cognitive dysfunction, epilepsy, neuropathic pain, inflammatory pain, Parkinson's disease, multiple sclerosis, stroke and sleep disorders including narcolepsy;
- 25 psychiatric disorders including schizophrenia, attention deficit hyperactivity disorder, depression and addiction; and other diseases including obesity, asthma, allergic rhinitis, nasal congestion, chronic obstructive pulmonary disease and gastro-intestinal disorders.

- 30 Thus the invention also provides a compound of formula (I) or a pharmaceutically acceptable salt thereof, for use as a therapeutic substance in the treatment or prophylaxis of the above disorders, in particular neurodegenerative disorders including Alzheimer's disease.

- 35 The invention further provides a method of treatment or prophylaxis of the above disorders, in mammals including humans, which comprises administering to the sufferer a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

In another aspect, the invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in the treatment of the above disorders.

- 5 When used in therapy, the compounds of formula (I) are usually formulated in a standard pharmaceutical composition. Such compositions can be prepared using standard procedures.

Thus, the present invention further provides a pharmaceutical composition for use in the treatment of the above disorders which comprises the compound of formula (I) or a
10 pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

The present invention further provides a pharmaceutical composition which comprises the compound of formula (I) or a pharmaceutically acceptable salt thereof and a
15 pharmaceutically acceptable carrier.

A pharmaceutical composition of the invention, which may be prepared by admixture, suitably at ambient temperature and atmospheric pressure, is usually adapted for oral, parenteral or rectal administration and, as such, may be in the form of tablets, capsules, oral liquid preparations, powders, granules, lozenges, reconstitutable powders, injectable or infusible solutions or
20 suspensions or suppositories. Orally administrable compositions are generally preferred.

Tablets and capsules for oral administration may be in unit dose form, and may contain conventional excipients, such as binding agents, fillers, tableting lubricants, disintegrants and acceptable wetting agents. The tablets may be coated according to methods well known in
25 normal pharmaceutical practice.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspension, solutions, emulsions, syrups or elixirs, or may be in the form of a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain
30 conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), preservatives, and, if desired, conventional flavourings or colorants.

For parenteral administration, fluid unit dosage forms are prepared utilising a compound of the invention or pharmaceutically acceptable salt thereof and a sterile vehicle. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions, the compound can be dissolved for injection and filter sterilised before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, preservatives and buffering agents are dissolved in the vehicle. To enhance the
35 stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially the same manner, except that the compound is suspended in the vehicle instead of being dissolved, and sterilisation cannot be
40 accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before

suspension in a sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

- 5 The composition may contain from 0.1% to 99% by weight, preferably from 10 to 60% by weight, of the active material, depending on the method of administration. The dose of the compound used in the treatment of the aforementioned disorders will vary in the usual way with the seriousness of the disorders, the weight of the sufferer, and other similar factors. However, as a general guide suitable unit doses may be 0.05 to 1000 mg, more suitably 1.0 to 200 mg, and such unit doses may be administered more than once a day, for example two or three a day. Such therapy may extend for a number of weeks or months.

The following Descriptions and Examples illustrate the preparation of compounds of the invention.

15 **Description 1**

1-Bromo-3-(4-chlorosulfonylphenoxy)propane (D1)

- A stirred solution of 3-bromo-1-phenoxypropane (4.3g) in chloroform (20ml) at -5°C was treated dropwise with a solution of chlorosulfonic acid (2.66ml) in chloroform keeping the temperature below 0°C . The reaction was stirred for 5 min then allowed to gain rt and stirred for 4 days. The mixture was poured onto ice and allowed to gain rt. The organic layer was collected, washed with water (3x20ml), saturated brine (20ml), dried (MgSO_4) and evaporated to give the title compound (D1) (1.9g). ^1H NMR δ (CDCl_3): 7.98 (2H, d, $J=8.8\text{Hz}$), 7.05 (2H, d, $J=8.8\text{Hz}$), 4.24 (2H, t, $J=5.8\text{Hz}$), 3.61 (2H, t, $J=5.8\text{Hz}$), 2.37 (2H, m).

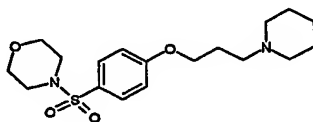
25 **Description 2**

4-[4-(3-Bromopropoxy)benzenesulfonyl]morpholine (D2)

- A solution of 1-bromo-3-(4-chlorosulfonylphenoxy)propane (D1) (200mg) in DCM (5ml) was treated with morpholine (0.14ml) and stirred for 1h. The solution was chromatographed (silica, step gradient 15 to 30% EtOAc in light petroleum $40^{\circ}\text{--}60^{\circ}$) to give the title compound (D2) (99mg). MS electrospray (+ion) 365 (MH^+). ^1H NMR δ (CDCl_3): 7.69 (2H, d, $J=9\text{Hz}$), 7.02 (2H, d, $J=9\text{Hz}$), 4.19 (2H, t, $J=5.8\text{Hz}$), 3.74 (4H, m), 3.61 (2H, t, $J=5.8\text{Hz}$), 2.99 (4H, m), 2.36 (2H, m).

Example 1

- 35 **N-[4-(3-Piperidin-1-ylpropoxy)benzenesulfonyl]morpholine hydrochloride (E1)**

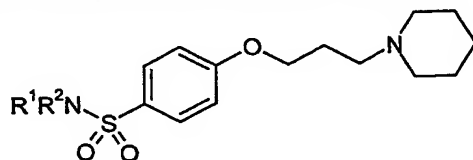


- 40 A solution of 4-[4-(3-bromopropoxy)benzenesulfonyl]morpholine (D2) (96mg) in 1-butanol (5ml) and piperidine (0.22ml) was heated at 100°C for 16h, cooled to rt and evaporated. The residue was redissolved in EtOAc (10ml), washed with saturated sodium hydrogen carbonate

solution (5ml), water (3x5ml), dried (MgSO₄) and evaporated. The residue was redissolved in DCM and treated with excess hydrogen chloride (4M solution in dioxan) and then concentrated to yield the title compound (E1) (75mg). MS electrospray (+ion) 369 (MH⁺). ¹H NMR δ (DMSO-d₆): 10.21 (1H, s), 7.68 (2H, d, J=8.8Hz), 7.18 (2H, d, J=8.8Hz), 4.18 (2H, t, J=6Hz), 3.62 (2H, m), 3.44 (2H, m), 3.17 (2H, m), 2.84 (6H, m), 1.30-1.85 (6H, m).

Examples 2-6 (E2-6)

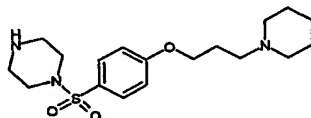
Examples 2-6 were prepared from the appropriate amine using an analogous method to that described in Description 2 (D2) followed by Example 1 (E1). All compounds displayed ¹H NMR and mass spectral data that were consistent with structure.



Example No	R ¹ R ² N	Mass Spectrum
E2		367 [M+H] ⁺
E3		443 [M+H] ⁺
E4		401 [M+H] ⁺
E5		401 [M+H] ⁺
E6		444 [M+H] ⁺

Example 7

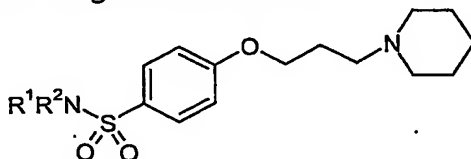
N-[4-(3-Piperidin-1-ylpropoxy)benzenesulfonyl]piperazine dihydrochloride (E7)

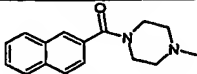
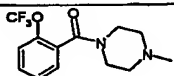


The title compound (E7) was prepared using an analogous method to that described in Description 2 (D2) followed by Example 1 (E1) by treating N-Boc piperazine with 1-bromo-3-(4-chlorosulfonylphenoxy)propane followed by reaction with piperidine. Subsequent deprotection with HCl afforded the dihydrochloride salt. MS electrospray (+ion) 368 (MH⁺).

Examples 8-9 (E8-9)

Examples 8-9 were prepared from Example 7 (E7) by treatment with the appropriate acid chloride in the presence of triethylamine using DCM as solvent..



Example No	R ¹ R ² N	Mass Spectrum
E8		522 [M+H] ⁺
E9		556 [M+H] ⁺

Abbreviations

	Boc	tertbutoxycarbonyl
5	EtOAc	ethyl acetate
	h	hour
	DCM	dichloromethane
	MeOH	methanol
	rt	room temperature
10	DCC	dicyclohexylcarbodiimide
	DMF	dimethylformamide

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Biological Data

A membrane preparation containing histamine H3 receptors may be prepared in accordance with the following procedures:

(i) Generation of histamine H3 cell line

The histamine H3 cDNA was isolated from its holding vector, pCDNA3.1 TOPO (InVitrogen), by restriction digestion of plasmid DNA with the enzymes BamH1 and Not-1 and ligated into the inducible expression vector pGene (InVitrogen) digested with the same enzymes. The GeneSwitch™ system (a system where in transgene expression is switched off in the absence of an inducer and switched on in the presence of an inducer) was performed as described in US Patent nos: 5,364,791; 5,874,534; and 5,935,934. Ligated DNA was transformed into competent DH5α *E. coli* host bacterial cells and plated onto Luria Broth (LB) agar containing Zeocin™ (an antibiotic which allows the selection of cells expressing the *sh ble* gene which is present on pGene and pSwitch) at 50μg ml⁻¹. Colonies containing the re-ligated plasmid were identified by restriction analysis. DNA for transfection into mammalian cells was prepared from 250ml cultures of the host bacterium containing the pGeneH3 plasmid and isolated using a DNA preparation kit (Qiagen Midi-Prep) as per manufacturers guidelines (Qiagen).

CHO K1 cells previously transfected with the pSwitch regulatory plasmid (InVitrogen) were seeded at 2x10⁶ cells per T75 flask in Complete Medium, containing Hams F12 (GIBCOBRL, Life Technologies) medium supplemented with 10% v/v dialysed foetal bovine serum, L-glutamine, and hygromycin (100μg ml⁻¹), 24 hours prior to use. Plasmid DNA was transfected into the cells using Lipofectamine plus according to the manufacturers guidelines (InVitrogen).

48 hours post transfection cells were placed into complete medium supplemented with 500µg ml⁻¹ Zeocin™.

10-14 days post selection 10nM Mifepristone (InVitrogen), was added to the culture medium to induce the expression of the receptor. 18 hours post induction cells were detached from the flask using ethylenediamine tetra-acetic acid (EDTA; 1:5000; InVitrogen), following several washes with phosphate buffered saline pH 7.4 and resuspended in Sorting Medium containing Minimum Essential Medium (MEM), without phenol red, and supplemented with Earles salts and 3% Foetal Clone II (Hyclone). Approximately 1x 10⁷ cells were examined for receptor expression by staining with a rabbit polyclonal antibody, 4a, raised against the N-terminal domain of the histamine H3 receptor, incubated on ice for 60 minutes, followed by two washes in sorting medium. Receptor bound antibody was detected by incubation of the cells for 60 minutes on ice with a goat anti rabbit antibody, conjugated with Alexa 488 fluorescence marker (Molecular Probes). Following two further washes with Sorting Medium, cells were filtered through a 50µm Filcon™ (BD Biosciences) and then analysed on a FACS Vantage SE Flow Cytometer fitted with an Automatic Cell Deposition Unit. Control cells were non-induced cells treated in a similar manner. Positively stained cells were sorted as single cells into 96-well plates, containing Complete Medium containing 500µg ml⁻¹ Zeocin™ and allowed to expand before reanalysis for receptor expression via antibody and ligand binding studies. One clone, 3H3, was selected for membrane preparation.

(ii) Membrane preparation from cultured cells

All steps of the protocol are carried out at 4°C and with pre-cooled reagents. The cell pellet is resuspended in 10 volumes of buffer A2 containing 50mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (pH 7.40) supplemented with 10e-4M leupeptin (acetyl-leucyl-leucyl-arginal; Sigma L2884), 25µg/ml bacitracin (Sigma B0125), 1mM ethylenediamine tetra-acetic acid (EDTA), 1mM phenylmethylsulfonyl fluoride (PMSF) and 2x10e-6M pepstain A (Sigma). The cells are then homogenised by 2 x 15 second bursts in a 1 litre glass Waring blender, followed by centrifugation at 500g for 20 minutes. The supernatant is then spun at 48,000g for 30 minutes. The pellet is resuspended in 4 volumes of buffer A2 by vortexing for 5 seconds, followed by homogenisation in a Dounce homogeniser (10-15 strokes). At this point the preparation is aliquoted into polypropylene tubes and stored at -70°C.

Compounds of the invention may be tested for *in vitro* biological activity in accordance with the following assays:

(I) Histamine H3 binding assay

For each compound being assayed, in a white walled clear bottom 96 well plate, is added:-

- (a) 10µl of test compound (or 10µl of iodophenpropit (a known histamine H3 antagonist) at a final concentration of 10mM) diluted to the required concentration in 10% DMSO;
- (b) 10µl ¹²⁵I 4-[3-(4-iodophenylmethoxy)propyl]-1H-imidazolium (iodoproxyfan) (Amersham; 1.85MBq/µl or 50µCi/ml; Specific Activity ~2000Ci/mmol) diluted to 200pM in assay buffer (50mM Tris(hydroxymethyl)aminomethane buffer (TRIS) pH 7.4, 0.5mM ethylenediamine tetra-acetic acid (EDTA)) to give 20pM final concentration; and

(c) 80µl bead/membrane mix prepared by suspending Scintillation Proximity Assay (SPA) bead type WGA-PVT at 100mg/ml in assay buffer followed by mixing with membrane (prepared in accordance with the methodology described above) and diluting in assay buffer to give a final volume of 80µl which contains 7.5µg protein and 0.25mg bead per well – mixture was pre-mixed at room temperature for 60 minutes on a roller.

The plate is shaken for 5 minutes and then allowed to stand at room temperature for 3-4 hours prior to reading in a Wallac Microbeta counter on a 1 minute normalised tritium count protocol. Data was analysed using a 4-parameter logistic equation.

10 (II) Histamine H3 functional antagonist assay

For each compound being assayed, in a white walled clear bottom 96 well plate, is added:-

(a) 10µl of test compound (or 10µl of guanosine 5'- triphosphate (GTP) (Sigma) as non-specific binding control) diluted to required concentration in assay buffer (20mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) + 100mM NaCl + 10mM MgCl₂, pH7.4 NaOH);

(b) 60µl bead/membrane/GDP mix prepared by suspending wheat germ agglutinin-polyvinyltoluene (WGA-PVT) scintillation proximity assay (SPA) beads at 100mg/ml in assay buffer followed by mixing with membrane (prepared in accordance with the methodology described above) and diluting in assay buffer to give a final volume of 60µl which contains 10µg protein and 0.5mg bead per well – mixture is pre-mixed at 4°C for 30 minutes on a roller and just prior to addition to the plate, 10µM final concentration of guanosine 5' diphosphate (GDP) (Sigma; diluted in assay buffer) is added;

The plate is incubated at room temperature to equilibrate antagonist with receptor/beads by shaking for 30 minutes followed by addition of:

(c) 10µl histamine (Tocris) at a final concentration of 0.3µM; and

(d) 20µl guanosine 5' [γ 35-S] thiotriphosphate, triethylamine salt (Amersham; radioactivity concentration = 37kBq/µl or 1mCi/ml; Specific Activity 1160Ci/mmol) diluted to 1.9nM in assay buffer to give 0.38nM final.

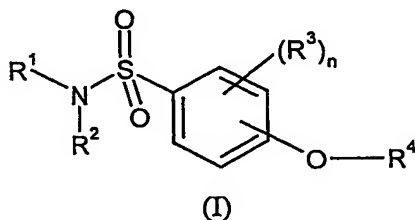
The plate is then incubated on a shaker at room temperature for 30 minutes followed by centrifugation for 5 minutes at 1500 rpm. The plate is read between 3 and 6 hours after completion of centrifuge run in a Wallac Microbeta counter on a 1 minute normalised tritium count protocol. Data is analysed using a 4-parameter logistic equation. Basal activity used as minimum i.e. histamine not added to well.

35 Results

The compounds of Examples E1-E9 were tested in the histamine H3 functional antagonist assay and exhibited pK_b values >7.5.

CLAIMS:

1. A compound of formula (I) or a pharmaceutically acceptable salt thereof:



wherein:

NR¹R² represents an N-linked -heterocyclyl, -heterocyclyl-X-aryl, -heterocyclyl-X-heteroaryl, -heterocyclyl-X-heterocyclyl, -heteroaryl, -heteroaryl-X-aryl, -heteroaryl-X-heteroaryl or -

heteroaryl-X-heterocyclyl group;

wherein said aryl, heteroaryl and heterocyclyl groups of NR¹R² may be optionally substituted by one or more (eg. 1, 2 or 3) substituents which may be the same or different, and which are selected from the group consisting of halogen, hydroxy, cyano, nitro, oxo, haloC₁₋₆ alkyl, polyhaloC₁₋₆ alkyl, haloC₁₋₆ alkoxy, polyhaloC₁₋₆ alkoxy, C₁₋₆ alkyl, C₁₋₆ alkoxy, arylC₁₋₆ alkoxy,

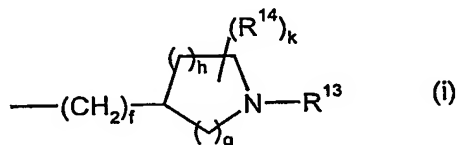
C₁₋₆ alkylthio, C₁₋₆ alkoxyC₁₋₆ alkyl, C₃₋₇ cycloalkylC₁₋₆ alkoxy, C₁₋₆ alkanoyl, C₁₋₆ alkoxycarbonyl, arylC₁₋₆ alkyl, heteroarylC₁₋₆ alkyl, C₁₋₆ alkylsulfonyl, C₁₋₆ alkylsulfinyl, C₁₋₆ alkylsulfonyloxy, C₁₋₆ alkylsulfonylC₁₋₆ alkyl, arylsulfonyl, arylsulfonyloxy, arylsulfonylC₁₋₆ alkyl, aryloxy, C₁₋₆ alkylsulfonamidoC₁₋₆ alkyl, C₁₋₆ alkylamidoC₁₋₆ alkyl, arylsulfonamido, arylaminosulfonyl, arylsulfonamidoC₁₋₆ alkyl, arylcarboxamidoC₁₋₆ alkyl, aroylC₁₋₆ alkyl, arylC₁₋₆ alkanoyl, or a group -NR¹⁵R¹⁶, -CONR¹⁵R¹⁶, -NR¹⁵COR¹⁶, -NR¹⁵SO₂R¹⁶ or -SO₂NR¹⁵R¹⁶, wherein R¹⁵ and R¹⁶ independently represent hydrogen or C₁₋₆ alkyl;

X represents a bond, CO, NHCO or CONH;

R³ represents halogen, C₁₋₆ alkyl, C₁₋₆ alkoxy, cyano, amino or trifluoromethyl;

n is 0, 1 or 2;

R⁴ represents -(CH₂)_q-NR¹¹R¹² or a group of formula (i):



wherein q is 2, 3 or 4;

R¹¹ and R¹² independently represent C₁₋₆ alkyl or together with the nitrogen atom to which they are attached represent an N-linked heterocyclic group optionally substituted by one or two R¹⁷ groups;

R¹³ represents hydrogen, C₁₋₆ alkyl, C₃₋₈ cycloalkyl, -C₁₋₆ alkyl-aryl or heterocyclyl;

R¹⁴ and R¹⁷ independently represent halogen, C₁₋₆ alkyl, haloC₁₋₆ alkyl, OH, diC₁₋₆ alkylamino or C₁₋₆ alkoxy;

f and k independently represent 0, 1 or 2;

g is 0, 1 or 2 and h is 0, 1, 2 or 3, such that g and h cannot both be 0;

or solvates thereof.

2. A compound according to claim 1 which is a compound of formula E1-E9 or a pharmaceutically acceptable salt thereof.

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3. A compound according to claim 1 or claim 2 for use in therapy.

4. A compound according to claim 1 or claim 2 for use in the treatment of Alzheimer's disease.

10

5. A pharmaceutical composition which comprises a compound according to claim 1 or claim 2 and a pharmaceutically acceptable carrier or excipient.

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PCT Application
EP0311649



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